

WVU IACUC Policy and Guidelines:

Breeding and Weaning of Mice and Rats

Purpose

This document establishes the parameters for appropriate rodent (mouse and rat) breeding activities under the WVU animal care and use program. It applies to all research personnel who list breeding activities in mice or rats on an approved animal use protocol. Exceptions to the policy or guidelines require prior IACUC approval. Any recurring issues with rodent breeding colony management should be brought to the attention of the IACUC and/or OLAR.

Background

The IACUC's role for oversight includes:

- 1) Evaluating the need for a breeding colony based on scientific justification of animal numbers
- 2) Approving all procedures related to the breeding process
- 3) Ensuring a mechanism for tracking animal numbers
- 4) Evaluating the standards of care and welfare for the animals in the breeding colony to ensure they are consistent with current policies and regulations

General Information

Optimal reproductive age span: mice 2-10 months; rats 2-15 months

Estrous cycle: 4-5 days

Gestation: mice 18-21 days; rats 21-23 days

Weaning age: ≥ 19 days old, usually 21 days, can be up to 28 days if approved by the IACUC (see below – Exceptions)

Postpartum estrus: a period within 24 hours after parturition when females are fertile and can conceive.

After this period, they are not fertile until the pups' weaning age (usually ~ 21 days)

Adult: defined as 6 weeks of age or older, based on the average age of sexual maturity

Policy

- Breeding of animals **must** be scientifically justified and described in an IACUC-approved animal use protocol. Number of animals produced during breeding should be tracked and kept to the minimum number required to meet scientific goals.
- Breeding fecundity should be monitored closely and females retired when litter size drops or pups have not been produced in 2 months.
- When using inbred strains, consider refreshing lines every ten generations to avoid creation of a new substrain and development of deleterious genotypic and phenotypic traits.
- Only one adult male rodent is permitted in a breeding cage.
- Female rodents **must** be at least six weeks old before they are placed with a sexually mature adult male for breeding. The only exception to this is superovulation of 3-4-week-old female mice for collection of zygotes or embryos. Super-ovulated females should not be allowed to go to term.
- Breeding Males **CANNOT** be re-housed together after they have been separated for breeding purposes, due to fighting.
- Common breeding setups in biomedical research include:

- Pairs: 1 female: 1 male
 - Trios: 2 females: 1 male
 - Harem: 3 females: 1 male. The need of this breeding setup must be scientifically justified in protocol and close attention paid to removing animal when visually pregnant.
 - Intensive breeding: When pairs remain together and females produce back to back litters due to post-partem estrus.
- Guide housing requirements (see IACUC policy: Biomedical Research- Primary Enclosure Space Density):
 - Mouse female + litter: 51 sq inches
 - Rat female + litter: 124 sq inches
 - Breeding cages cannot exceed the guide cage density requirements.
 - Two or more litters are not to be present at the same time in a cage unless approved by the IACUC and special caging is available. This stipulation includes litters of similar or disparate ages.
 - Ventilated cages used at WVU will permit a mouse pair + litter or female rat + litter.
 - Trio and Harem breeding setups must remove one or more female(s) when visually pregnant PRIOR to giving birth.
 - Pregnant female rats should be separated into their own cage when visually pregnant PRIOR to giving birth.
 - If mouse breeding pairs remain together, the female will likely become pregnant soon after birth due to postpartum estrus. If this occurs, the older pups MUST be weaned by day 21 (no delayed weaning) or within 24 hours of the new litter being born (preferably before). Males should be removed when females are visually pregnant if delayed weaning is needed or intensive breeding is not desired.

The Office of Laboratory Animal Resources (OLAR) veterinary staff can provide training and additional information on best practices for rodent breeding to research staff, upon request.

Guidelines

A. Colony Management

1. General Information

- a. Breeding cages should be regularly monitored to ensure the well-being of adults and neonates, appropriate cage environment and colony breeding performance. Changes should be made when necessary (i.e. separating mice, moving animals to a clean cage, retiring breeders, etc.).
- b. Animal usage records should be maintained to track offspring and their ultimate disposition. Suggested information would include sire/dam #, date and # born, date and # weaned and final disposition (i.e. euthanized, died, used in experiment, transferred to another protocol, or retained for breeding).
- c. Record keeping and colony management practices should demonstrate efforts to utilize animals in ways that conserve genetic traits and are not wasteful.
- d. Investigators maintaining breeding colonies exclusively to preserve a genetic line of rodents should consider other strategies such as cryopreservation of ova, sperm and/or embryos.

- e. When newborn pups are found, the date of birth **must** be recorded on the cage card, and a white [DO NOT CHANGE] cling placed on the cage, per OLAR's Mouse or Rat Husbandry SOP.
 - *OLAR staff typically take care of this, but research staff should follow the same practice if they note pups.*

2. Breeding Schemes – MICE

- a. Monogamous pair (1 male, 1 female) in a standard housing cage. Male is not necessarily separated when the female becomes pregnant or delivers the pups. Litters are born approximately 21 days apart. The three-week-old litter **must** be weaned prior to the birth of the new litter.
- b. Breeding trios (1 male, 2 females) in a standard housing cage. Pregnant females **must** be separated prior to parturition, and only 1 litter of pups with up to 2 adults may remain in the cage after pups are born.
- c. Harem breeding (1 male, 3-4 females) in a standard housing cage. Pregnant females **must** be separated prior to parturition, and only 1 litter of pups with up to 2 adults may remain in the cage after pups are born.

3. Breeding Schemes – RATS

- a. Any breeding schemes used must be described under the breeding section in an IACUC-approved protocol. Consult with OLAR staff to determine options based on type of cages used.

4. Overcrowded Cages

- a. A cage is considered overcrowded if a new litter is born before the older litter from the same female is weaned. If postpartum estrus is used, the first litter **must** be weaned by 21 days of age to prevent the presence of two litters in a cage.
- b. **MICE**
 - i. Adhere to above housing density standards to prevent overcrowding.
 - ii. Maximum of 5 adult mice may be housed per standard mouse cage. If there are more than five young mice in a cage, the date of birth **must** be written on the cage card, to indicate they are not adults. If the age of the mice is unclear, they **must** be housed at a maximum of 5 per cage.
- c. **RATS** – the number of rats allowed per cage will vary depending on the type of cages used, and the size/weight of the rats. Consult with OLAR staff to determine maximum housing density.

5. Weaning

- a. Litters should be weaned by postnatal day 21, or before the birth of a second litter, unless there is an approved exception in the IACUC protocol (see below – Exceptions).
- b. Weanling animals should be placed in a clean, standard housing cage with food and water and sorted by sex. Regardless of the caging system, a fresh water bottle should be provided to newly weaned rodents.
- c. Food and water **must** be accessible to weaned animals. It is helpful to provide a few feed pellets and/or moist feed in a petri dish on the floor for easy access at the time of weaning.

- d. If cages of newly weaned pups need additional observation, contact OLAR staff for recommendations on how to identify the cages.
- e. OLAR staff will wean the litter if research staff does not perform this by postnatal day 22, or when a second new litter is born. **There is a charge for this service.** Continued failure of the research staff to wean in a timely manner will be considered a non-compliance issue and is reportable to the IACUC.

6. Cross Fostering

- a. When a dam can no longer care for a litter, fostering the litter to another lactating dam may be an option. Examples of when to consider fostering:
 - i. Dam dies
 - ii. Dam illness
 - iii. Lactation concerns (mammary glands not full, no milk spots present in litter)
 - iv. Cannibalism
- b. To foster a litter
 - i. Discuss the circumstances and fostering plan with the PI. Obtain PI's permission before proceeding.
 - ii. Work with the PI to identify a reasonable foster dam:
 - Ideally, the foster dam should be caring for a litter that is the approximate age of the litter to be fostered (within 1-week). The foster dam should have a history of being a good mother or be a strain known to be good mothers.
 - If possible, the foster dam should be a different coat color than the litter to be fostered to reduce difficulty in identifying the origin of the pups.
 - The total pup number should not exceed the maximum number of pups typical for the foster dam strain (if litters are large, some pups may need to be euthanized to reduce litter size or the litter may need to be divided among multiple foster dams)
 - iii. If males are housed with the foster dams, they must be removed and placed into a separate cage
 - iv. All adult females should be temporarily removed from the home cage.
 - v. The foster litter should be placed into the nest with the litter currently in the cage. The foster litter should be gently rubbed with the dirty bedding and nesting material to obtain the scent from the foster dam and litter.
 - vi. Hold the foster dam over all pups and have her urinate on the combined litter. Gently rub the urine onto the new foster litter.
 - vii. Place the adult female(s) back into foster cage.
 - viii. Place the cage in a quiet location with minimal disturbances. Check on the cage a few times over the next 2-3 hours. Look into the cage and monitor for signs of rejection, but do not open unless necessary.
 - The foster female should gather the new litter into the nest, cleaning the pups, and nurse. This is a good sign that the pups will be accepted.
 - If the foster dam removes the foster pups from the nest, drags them around the cage, or cannibalizes them, remove the pups immediately and attempt to foster with a new female.

B. Exceptions

1. Extended weaning date:

If a litter of normal weaning age is judged to be not ready to wean, veterinary staff should be consulted. Examples include small pup size, slow growth rate, and inability to access food or water.

Delayed weaning up to seven days can be approved by veterinary staff. For strains where extended weaning is required, the male should be removed before birth and until after weaning is complete to avoid impregnation of the female during postpartum estrus. Cages are typically placed on clinical call when extending the weaning period in consultation with veterinary staff.

2. Allomothering:

In instances where allomothering (“aunting”) by a second female is known (by presenting data to that effect) to increase survival of pups, harem breeding may extend to nursing, if justified and approved by the IACUC. Some specialized genetically modified lines may require more than 2 adults and 1 litter in the breeding cage to facilitate adequate production. Breeding records demonstrating poor breeding *must* first be reviewed with the facility veterinarian and scientific justification submitted to the IACUC for approval. Larger caging may be necessary to comply with housing density recommendations in the *Guide*.

3. Requests for a perpetual exception to extend weaning date *must* be submitted to the IACUC. If approved, OLAR should also be contacted regarding how to identify the weaning exception at the cage level.

Some reasons for extending weaning date include-

- a. Experimental design, e.g. studying psychological imprinting of pups
- b. Need to prevent loss of litters due to cannibalism
- c. Need to extend nurturing time in strains that fail to thrive

C. Counting Animals

- *OLAR staff typically take care of this, but research staff should follow the same procedures if they remove pups before they are counted by OLAR staff.*

References

- 1. [Guide for the Care and Use of Laboratory Animals](#), National Research Council, 2011.
- 2. [Breeding Strategies for Maintaining Colonies of Laboratory Mice](#), The Jackson Laboratory.